

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
31 May 2001 (31.05.2001)

PCT

(10) International Publication Number
WO 01/37870 A1

(51) International Patent Classification: **A61K 39/395,**
G01N 33/53, 33/577, A61P 37/02

(GB). LONDEI, Marco [IT/GB]; 3 Prebend Mansions,
Chiswick High Road, London W4 2LU (GB).

(21) International Application Number: PCT/GB00/04511

(74) Agents: ELSY, David et al.; Withers & Rogers, Goldings
House, 2 Hays Lane, London SE1 2HW (GB).

(22) International Filing Date:

27 November 2000 (27.11.2000)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

9927757.6 25 November 1999 (25.11.1999) GB

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): THE
MATHILDA AND TERENCE KENNEDY INSTI-
TUTE OF RHEUMATOLOGY [GB/GB]; 1 Aspenlea
Road, Hammersmith, London W6 8LH (GB).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): MAURI, Claudia
[IT/GB]; 26 Brooksby Street, London N1 1HA (GB).
MARS, Leonaredus, Theodorus [NL/GB]; c/o The
Mathilda and Terence Kennedy Institute of Rheumatol-
ogy, 1 Aspenlea Road, Hammersmith, London W6 8LH

Published:

— With international search report.

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: TREATMENT OF AUTOIMMUNE DISEASES BY AN AGONISTIC CD40-BINDING PROTEIN

(57) Abstract: The application discloses methods of treating autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, diabetes myellitis or multiple sclerosis comprising administering an agonistic CD40 binding protein such as an anti-CD40 antibody. Preferably the anti-CD40 antibody is FGK45 or 3\23.

WO 01/37870 A1

TREATMENT OF AUTOMMUNE DISEASES BY AN AGONISTIC CD40-BINDING PROTEIN

The invention relates to the treatment of autoimmune diseases such as rheumatoid arthritis, with CD40 binding proteins such as anti-CD40 antibodies.

There is compelling evidence that T lymphocytes drive immuno-mediated pathologies, such as autoimmune diseases and transplant rejection, consequentially much effort has been focused in recent years on devising successful strategies able to treat these pathologies by controlling the expansion of pathogenic T cells. The increasing knowledge of the basic mechanisms governing T cell activation has allowed the emergence of a novel generation of compounds able to modulate T cell function and to induce a state of immunological tolerance permitting transplant acceptance and amelioration of autoimmune diseases. For instance the abrogation of T cell co-stimulation by blocking CD28 engagement, one of the essential second signals involved in T cell activation¹, with CTLA4-Ig has been successful in many models²⁻⁴. Moreover, in humans, CTLA4Ig has been recently shown to provide very promising results when used in long term bone marrow allograft acceptance⁵. Another surface molecule CD154, formerly called CD40L, has been extensively targeted for immunotherapeutic purposes due to the key role that this molecule plays in modulating immune responses principally via the engagement of CD40 on B lymphocytes on dendritic cells (DC)⁶. Many studies have indicated the great therapeutic potential of targeting CD154. For instance anti-CD154 monoclonal antibody (mAb) prevents the induction of autoimmune disorders in many animal models⁷⁻¹⁰, and also ameliorates established diseases (R-EAE)¹¹. Most remarkably, disrupting the CD154-CD40 interaction using an anti-CD154 mAb *in vivo*, permitted practically indefinite kidney graft acceptance in non-human primates thus moving this approach one step closer to its clinical application¹². Intriguingly, the inventors have recently observed that down-modulation of the CD154 expression has a key role even in other well established therapeutic approaches, such as non depleting anti-CD4 mAb therapy, thus further stressing the relevance of CD154

modulation in immuno-mediated pathologies. Intuitively the *in vivo* use of agonistic anti-CD40 mAbs, mimicking the action of CD154, strongly boosts immune responses¹³. More recent reports have shown that *in vivo* administration of agonistic anti-CD40 mAb can have a key role in promoting protective immune responses against B cell lymphomas¹⁴, as well as promoting antigen specific immunotherapy for cancer^{15,16}. The inventors have also identified the surprising ability of agonistic anti-CD40 mAb to control arthritis when administered during disease induction.

Puzzled by the apparent contrast between the widely described *in vivo* adjuvant action of agonistic anti-CD40 mAb and the inventor's preliminary findings, the inventors have explored the therapeutic potential and the possible mechanisms of action of agonistic anti-CD40 mAb in a model for CCIA (Chronic Collagen Induced Arthritis)¹⁷. They have reported that agonistic anti-CD40 mAb have a remarkable therapeutic effect on the development of arthritis, thus indicating their potential clinical use to control chronic inflammatory conditions of autoimmune origin.

First aspect of the invention provides use of an agonistic CD40 binding proteins, such as anti-CD40 antibody, in the manufacture of a medicament to treat an autoimmune disease.

The CD40 binding protein may also be a CD40 ligand. Such ligands are known in the art and are shown, for example, in WO 96/26735. Such ligands are expected to behave in a similar manner to the agonistic anti-CD40 antibodies.

Agonistic anti-CD40 antibodies are able to induce activation of CD40. For example, they mimic the action of natural CD40 ligands by activating CD40.

Methods of treatment using such binding proteins are also provided.

The term "pharmaceutically effective dose" means an amount of anti-CD40 antibody sufficient to ameliorate the autoimmune diseased state in an animal or patient. The dose required can be assessed using routine clinical trials. The typical dose of antibody will be

in the range of about $1\mu\text{g}/\text{kg}/\text{day}$ to $10\text{ mg}/\text{kg}/\text{day}$ of patient or animal body weight. Preferably, this dose is at least $0.01\text{ mg}/\text{kg}/\text{day}$.

Preferably the animal or patient is a mammal, especially a human.

The anti-CD40 antibody may be introduced into a patient by any methods known in the art. For example, the antibody may be delivered intramuscularly, intravenously or parenterally.

The anti-CD40 antibody may be a polyclonal antibody or a monoclonal antibody. Techniques for the production of polyclonal antibodies or monoclonal antibodies are well known in the art.

As used herein, the term "antibody" refers to intact molecules as well as fragments thereof, such as Fa , F(ab')_2 , and Fv which are capable of binding the CD40 protein. Intact CD40 polypeptide or fragments of CD40 may be used as the immunising antigen.

A natural ligand for CD40, such as CD154 (cD40L) may be used.

The anti-CD40 antibodies may be humanized, that is how amino acids replaced in the non-antigen binding regions in order to more closely resemble a human antibody, whilst still remaining the original binding ability. Methods for humanizing antibodies are well known in the art.

Preferably the antibody is a monoclonal antibody with functional characteristics when implemented in man of FGK45 or 3\23, or soluble CD154.

The autoimmune disease is preferably selected from rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and diabetes myllitus.

Antibodies are preferably administered after disease onset. That is, the antibody is used to treat the diseased state, rather than helping to prevent the disease.

A second aspect of the invention provides a method of studying an autoimmune disease comprising administering a pharmaceutically effective dose of an agonistic anti-CD40 antibody to an animal, sample of tissue or cell and observing a physiological change to the animal, sample of tissue or cell. Preferably such a method is carried out *in vitro* on an isolated cell, cultured cell or an isolated sample of tissue. This allows the effect of anti-CD40 antibodies on the cells to be studied. Preferably the cell, tissue, or antibody is diseased, that is it has characteristics of an autoimmune disease.

The antibodies and autoimmune diseases may be as defined above.

Another aspect of the invention provides an anti-CD40 antibody for use to treat an autoimmune disease. The anti-CD40 antibody and autoimmune disease may be as defined above.

Further aspects of the invention provides an anti-CD40 antibody in combination with a pharmaceutically effective carrier. Pharmaceutically effective carriers for antibodies are well known in the art.

Preferred embodiments of the invention will now be described with reference to the following figures:-

Figure 1

Stimulation of CD40, with an agonistic mAb, delays the onset and reduces the severity of CCIA.

Sex-matched TcR- β Tg mice, 8-12 weeks old, were immunised with 200 μ g/ml. of bovine Cii in CFA. Mice were treated over an 18 day period with a total of 14 injections of FGK45 (Δ) (300 μ g, $n = 10$), 3 λ 23 (O) (300 μ g, $n = 8$). The control group (\blacklozenge) consists of the pooled results of mice treated with either AFRC-MAC-1 (300 μ g, $n = 10$) or PBS (300 μ l, $n = 10$). Disease evolution was monitored daily. Results represent the mean clinical score of n mice \pm SEM and are representative of three individually performed experiments.

Figure 2**Agonistic anti-CD40 mAb treatment induces B cell expansion.**

Sex-matched TcR- β Tg mice, 8-12 weeks old, were immunised with 200 μ g/ml. of bovine collagen type II in CFA. Mice were treated from the day of immunisation for 8 days with daily injections of 300 μ l PBS (a), 300 μ g AFRC-MAC-1 (b), 300 μ g 3 λ 23 (c), 300 μ g FGK45 (d). Spleens removed from the animals were snap-frozen 8 days after immunisation, 5 μ m sections were stained with anti-B220 mAb. Isotype matched control mAb gave negative staining.

Figure 3**Therapy of CCIA with agonistic anti-CD40 mAb ameliorates established arthritis.**

Sex-matched TcR- β Tg mice, 8-12 weeks old, were immunised with 200 μ g/ml. of bovine collagen type II in CFA. Mice were treated daily from the day of clinical disease onset, a total of 10 injections of FGK45 (Δ) (300 μ g, n = 10), 3 λ 23 (O) (300 μ g, n = 10) were administered. The control group (\blacklozenge) consists of the pooled results of mice treated with either AFRCMAC-1 (300 μ g, n = 10) or PBS (300 μ l, n = 10). Clinical score (A) and paw thickness (B) were monitored daily. Results represent the mean of n mice \pm SEM and consist of the pooled data of two independently performed experiments.

Figure 4**Histological evaluation of anti-CD40 mAb therapy.**

Arthritis in the hind paws were stained with haematoxylin and eosin, and the joints erosion was scored as described in Materials and Methods. Joint representative of mouse treated with (a) PBS, (b) AFRC-MAC-1, (c) FGK45 and (d) 3 λ 23. The results are also shown as the percentage of all paws studied that were given the specified score (e).

Figure 5

Therapeutic administration of agonistic anti-CD40 mAb preferentially induces CII specific IgG1 over IgG2a immunoglobulin isotypes.

Mice were treated daily from disease onset with FGK45 (○), 3V23 (□), AFRC-MAC-1 or PBS, the latter two combined as control (■), for a total of 10 days. Peripheral blood serum was collected 12 days after disease onset. CII specific IgG2a and IgG1 titers were detected by ELISA. The results are represented as the mean fold increase of the CII specific IgG titer of FGK45 and 3V23 relative to the control CII specific IgG titer \pm SEM.

Figure 6

Agonistic anti-CD40 mAb therapy alters the cytokine profile of antigen specific activated T cells.

Individual spleens were collected from FGK45 (○)($n = 5$), 3V23 (□)($n = 5$) or AFRC-MAC-1 (■)($n = 5$) treated mice (treatment as described for figure 3). Single cell suspensions of each individual spleen were cultured at 5×10^6 cells/ml. in the presence of CII (a, b, c), or CII and anti-CD40 mAb (d, e, f) (indicated below graph), supernatants were analysed after 24 hrs. for IL-4 (b, e) and IL-5 (c, f)) or after 72 hrs. for IFN- γ (a, d). Results represent the mean cytokine expression of n independent splenocyte cultures obtained from individual mice \pm SEM (* $p < 0.05$, ** $p < 0.001$). Statistics in graphs d, e and f are relative to the values represented in graphs a, b and c respectively.

Figure 7

Stimulation of CD40, with an agonistic mAb, before immunisation does not alter the course of CIA.

Sex-matched TcR- β Tg mice, 8-12 weeks old, were treated with 5 daily injections of FGK45 (Δ)(300 μ g, $n = 5$), 3\23 (O) (300 μ g, $n = 5$). The control group (\blacklozenge) consists of the pooled results of mice treated with either AFRC-MAC-1 (300 μ g, $n = 5$) or PBS (300 μ l $n = 5$). On the last day of treatment the mice were immunised with 200 μ g/ml. of bovine collagen type II in CFA. Disease evolution was monitored daily. Results represent the mean clinical score of n mice \pm SEM.

Figure 8 shows the effect of AFRC Mac-1 monoclonal antibodies and anti-CD40 antibodies on anti-CII Ig G2a and anti CII Ig G1 production.

Figure 9 shows the effect of anti-CD40 on IL-12 expression on pathogenic/arthritisogenic splenocytes from mice *in vivo*.

MATERIALS AND METHODS

Mice.

The previously described heterozygous TcR- β Tg SWR/J mice TcR- β Tg¹⁷ was backcrossed into DBA/1 in order to derive the Chronic Collagen Induced Arthritis model³⁶, subsequent transgenic offspring was consistently backcrossed against DBA/1. Mice from generation N10-N15 typed for TcR- β Tg expression, were selected for the experiments.

Antibodies.

The treatment antibodies used were FGK45, rat IgG anti-mouse CD40³⁷ (kindly provided by Dr A. Rolink, Basel Institute for Immunology); 3 λ 23 (kindly provided by Dr D. Gray, Hammersmith Hospital, London), rat IgG2a anti-mouse CD40³⁸; AFRC MAC-1 (isotype control), rat IgG2a anti-dog chlamydomonas cell wall glycoprotein (European Collection of Animal Cell Culture, Salisbury, UK). The mAbs were purified from culture supernatants by affinity chromatography, using a staphylococcal protein G column (Bioprocessing, Durham, UK) and filter sterilised.

The mAbs used for FACS-analysis were against mouse CD3 FITC, mouse $\nu\beta$ 12 TcR biotinylated (screening), mouse B220 PE, and streptavidin-PE, all obtained from Pharmingen (San Diego, CA). Finally, the primary antibodies used for immunocytochemistry were anti-mouse B220 biotinylated and an isotype control rat IgG2a biotinylated, both obtained from Pharmingen (San Diego, CA).

The coating/detection antibody pairings used for ELISA are as follows, IL-2: JES6-1A12/JES6-5H4 (Pharmingen, San Diego, CA); IL-4: id11/BVD6-24G2 (ATCC and Pharmingen, San Diego, CA, respectively); IL-5: HB9897/HB10647 and IFN- γ (R4-6A2/XG1.2), ATCC, courtesy of Dr J. Abrams at DNAX (Palo Alto, CA).

Preparation of Collagen.

Bovine CII was purified and prepared as previously described³⁹. Bovine CII was solubilized by stirring overnight at 4°C in 0.1 M acetic acid, to be used for immunisation, or 0.05 mM Tris-HCL, 0.2 M NaCl pH 7.4 for *in vitro* stimulation of splenocyte cultures (SPC).

Induction and assessment of arthritis.

Sex-matched TcR- β Tg mice (8.12 weeks old) were immunised with 200 μ g of bovine CII emulsified in CFA (Difco Laboratories, Detroit, MI). The development of arthritis was assessed daily for the duration of the experiment. The clinical severity of arthritis was graded as follows, 0 = normal, 1 - slight swelling and/or erythema, 2 = pronounced oedematous swelling, 3 = pronounced oedematous swelling plus light joint rigidity, 4 = laxity. Each limb was graded, allowing a maximal clinical score of 16 for each animal. Swelling of hind paws was recorded with a pair of callipers. All clinical evaluations were performed in a blinded manner.

Immunotherapy.

Three experimental protocols have been performed. In the first set, TcR- β Tg mice were i.p. treated, from the day of CII in CFA immunisation, with either FGK45, 3 λ 23, AFRC MAC-1 or with PBS, with 14 daily doses over a period of 18 days. In the therapeutic approach, TcR- β Tg were treated i.p. with FGK45, 3 λ 23, AFRC or with PBS from the day of disease onset with a total of 14 i.p. injections over 18 days. The pre-immunisation treatment was performed by 5 daily i.p. injections with FGK45, 3 λ 23 or AFRC MAC-1 initiated 5 days prior to CII in CFA immunization. All antibodies were administered at 300 μ g/mouse.

Histological evaluation.

Hind paws were removed *post mortem* and fixed in 10% (w/v) buffered formalin and decalcified in 5% EDTA. The paws were subsequently embedded in paraffin, sectioned and stained with haematoxylin and eosin or safranin O. Arthritic changes in the ankle, the metatarsophalangeal joints, the proximal interphalangeal and the distal interphalangeal joints were scored blindly as normal (unaffected), mild (mild synovial hyperplasia), moderate (pannus formation and limited erosion), or severe (extended bone and cartilage erosion with loss of joint architecture).

Cytokine quantification.

Spleen were teased apart to make a single cells suspension and red blood cells were depleted with a red cell lysis buffer (Sigma Aldrich, Dorset, UK), washed and cultured in RPMI 1640 containing 10% (v/v) heat-inactivated FCS, 100 U/ml. penicillin, 100 µg/ml. streptomycin, 2×10^{-5} M 2-mercaptoethanol, and 20 mM L-glutamine. SP were cultured in 96-well plates (Nunc, Uxbridge, UK) at the density of 5×10^6 cells/ml. (200 µl/wells) in medium alone or with 50 µg/ml. of CII for 24 h. for IL-4 and IL-5 detection or for 72 hr. for IFN- γ . Supernatants were collected and analysed for cytokines were quantified by a sandwich ELISA as previously described ⁴⁰.

Serum collagen specific immunoglobulin quantification.

Anti-CII antibodies were quantified as previously described ⁴¹. Briefly, microtiter plates (Nunc, Uxbridge, UK) were coated overnight with 2 µg/ml. of native bovine CII dissolved in Tris buffer (0.05 M Tris, 0.2 M NaCl, pH 7.4), blocked with 2% BSA and incubated with serially diluted test sera. Bound IgG was detected by incubation with alkaline phosphatase-conjugated sheep anti-mouse IgG (the binding site, Birmingham, UK) followed by the substrate dinitrophenyl phosphate. Plates were washed at each individual step for at least 3 times with 0.001% Tween/PBS. The optical density was measured at 405 nm.

Cytochemistry.

TcR- β Tg mice were treated from the day of immunisation with 8 daily intraperitoneal injections of the indicated mAbs at 300 μ g/mouse:

Spleens were excised *post mortem* 8 days after immunisation, embedded in OCT compound (Raymond A. Lamb, London, UK) and 'snap-frozen' in liquid nitrogen/isopentane bath. Sections were obtained of 5 μ m and adhered to microscope slides (Merck Ltd, Leicester, UK). Wax-encircled sections were blocked with rat serum before being incubated for 1 hr. with the biotinylated primary antibodies followed by streptavidin labelled alkaline phosphatase (Vector, Burlingame, Ca). Enzyme localisation was visualised with the substrate fast-red according to the manufacturers' recommendations (DAKO, Carpinteria, Ca) and fixed in 2% formaldehyde for 20'. Finally, slides were washed in water and coverslipped with glycerol gelatin (Sigma Aldrich, Dorset, UK). Slides were washed at least 3 times in PBS at each step.

Statistical analysis.

For the statistical analysis of the data, the Mann-Whitney U test and the 2-way ANOVA were applied.

RESULTS

Engagement of CD40 during the induction phase of the autoimmune response reduces the severity of CCIA.

In another, unpublished, study the inventors unexpectedly observed that anti-CD40 mAb treated mice, like the anti-CD4 mAb treated animals, showed a significant reduction in disease severity. To expand our findings, two groups of TcR- β Tg mice were treated, from the day of immunisation, with two different agonistic anti-CD40 mAbs (FGK45 or 3 λ 23) which have been previously used by several groups for *in vivo* upregulation of immune responses^{14, 16, 18}. Agonistic anti-CD40 mAb, AFRC- MAC-1, (300 μ g/mouse daily) or PBS were administrated 14 times over an 18 day period from the day of immunisation.

Figure 1 shows the suppressive effects of the anti-CD40 mAb treatment on the evolution of the most severe (onset of the disease 13-14 as opposed to the usual day 19-20) form of CCIA, which we have observed in 1 out of 3 independent experiments. Even when administered in these experimental conditions both agonistic anti-CD40 mAb exerted their ability to control onset and to significantly reduce the severity of disease when compared with the results obtained from the control treated group (Figure 1 and Table 1).

Table 1.

GROUP	DAY OF ONSET	PAW THICKNESS (mm)			
		DAYS AFTER ONSET			
		DAY 2	DAY 4	DAY 6	DAY 8
Control	13.5 \pm 0.3	1.9 \pm 0.11	2.2 \pm 0.13	2.3 \pm 0.13	2.2 \pm 0.12
FGK45	18.3 \pm 1.5	1.9 \pm 0.08	1.9 \pm 0.11	2.0 \pm 0.14	1.9 \pm 0.17
3 λ 23	16.3 \pm 2.8	1.9 \pm 0.10	2.0 \pm 0.17	2.1 \pm 0.15	2.1 \pm 0.16

Effects of agonistic anti-CD40 mAb treatment on the induction of CCIA.

Sex-matched TcR- β Tg mice, 8-12 weeks old, were immunised with 200 μ g/ml. of bovine collagen type II in CFA. Mice were treated over an 18 day period with a total of 14 injections of FGK45 (300 μ g, n = 10), 3 λ 23 (300 μ g, n = 8). The control group consists of

the pooled results of mice treated with either AFRC-MAC-1 (300 μ g, $n = 10$) or PBS ($n = 10$). Disease evolution was monitored daily.

- a) Day of clinical onset of disease expressed as the mean of n mice \pm SEM.
- b) Spleens were removed on day 35 after immunisation, single cell suspensions of 5 pooled spleens were stained with FITC-anti-CD3 and PE-anti-B220 and analysed by flow cytometry. Ratio was calculated as number of B220⁺ cells over CD3⁺ cells.
- c) Paw swelling was measured for each inflamed hind paw. Results expressed as mean swelling of n inflamed paws \pm SEM.

All results are representative of three individually performed experiments.

In agreement with previous reports¹³ splenomegaly (data not shown) and an expansion of B lymphocytes (Table 1) were observed in response to *in vivo* administration of either agonistic anti-CD40 mAbs. The increase of the splenic B cell population was also confirmed, by immuno-histochemistry, in a parallel experiment 8 days after immunisation in the 323 and FGK45 treated mice. As shown in Figure 2, a clear increase in number of B220⁺ B cells was observed in the marginal zone of both anti-CD40 mAbs (2 d and e) treated animals compared to control group (2 a and B). Aspecific crossreactions were carefully excluded in each analysed sample by using an appropriate isotype control mAb (data not shown).

Anti-CD40 mAb therapy ameliorates established CCIA.

We have previously shown that the CCIA animal model is ideally suited to perform therapeutic studies on established autoimmune disease¹⁷, and we decided to investigate the effects of agonistic anti-CD40 mAb on the progression of established arthritis. In three independent experiments, at least 5 TcR- β Tg mice were treated from the day of clinical

onset with 10 daily injections (300 µg/mouse) of FGK45, 3\23, AFRC-MAC-1 or 300 µl/mouse of PBS, the latter two refereed as control groups.

As shown in Figure 3a both anti-CD40 mAbs showed a strong suppressive effect on the progression of disease, which was apparent within one day of the first administration. In fact, both 3\23 and FGK45 treated mice manifested a significantly reduced clinical severity of arthritis ($p = 0.0017$ and $p < 0.0001$, respectively) compared to the control treated group. The amelioration of arthritis was also supported by the significant inhibition of paw swelling measured in mice treated with either FGK45 ($p < 0.0001$) or 3\23 ($p < 0.0001$) compared to the results obtained in the control group (Figure 3b).

Histological features of anti-CD40 mAb therapy.

Blinded histological examination was carried out to determine whether the amelioration of disease in anti-CD40 mAb treated mice correlated with reduced joints damage. Histologically 61, 25% of the joints from the control group were severely damaged by the expansion of the synovial pannus, fibrin essudate and mononuclear cells, which accumulate in the synovial space (Figure 4a). Consequently, a complete loss of the bone architecture was also observed in half of these severely affected control joints (Figure 4b). In contrast to the results scored in the control group, only 17.6% of joints from 3\23 treated animals were severely damaged, but even in these severe cases the bone architecture was left intact. Moreover, 53% of joints were affected by a very mild arthritis (Figure 4c) with minimal signs of inflammation and a negligible accumulation of inflammatory cells. Finally, 12% of joints were scored as normal with no signs of the initiation of synovitis, thus indicating the powerful suppressive effect of this therapeutic approach on inflammatory processes. FGK45 treatment completely protects 24% of the joints, and 29% were only mildly affected showing little erosion of the cartilage (Figure 4d). The remainder of the joints showed bone erosion and accumulation of inflammatory cells. The histological analysis of all the joints studied is reported in Figure 4e.

Therapeutic administration of anti-CD40 mAb preferentially induces IgG1 CII specific immunoglobulins.

Anti-CII antibodies are a trademark of CIA (collagen-induced arthritis), and have been reported to play a pathogenic role in the evolution of arthritis¹⁹. In CIA, as in other models of autoimmune disease, a Th1 self-antigen specific response drives the immune response²⁰. Therefore, upon CII in CFA immunisation high levels of CII specific IgG2a antibodies, a Th1 dependent subclass²¹, are produced. The isotype subclasses are an accepted indicator of whether a dominant Th1 or Th2 response is mounted against CII in CIA. Thus the variation of the Th1 (IgG2a) or Th2 (IgG1) responses is generally monitored in mice after therapeutic manipulations to evaluate possible *in vivo* shifts from a pathogenic Th1 (IgG2a) to a potentially protective Th2 (IgG1) response^{22, 23}. Peripheral blood serum CII specific IgG1 and IgG2a levels were quantified 12 days after disease onset (Figure 5). Both agonistic anti-CD40 mAbs upregulated the CII specific IgG2a (Th1) levels by 29 (FGK45) and 72 (3\23) fold, relative to control treated mice. Critically, the levels of IgG1 (Th2) were amplified up to 46 fold for FGK45 and 260 fold in 3\23 treated mice. These data suggest that anti-CD40 mAb therapy would preferentially redirect the IgG2a/IgG1 CII specific antibody ratio in favour of the Th2 controlled IgG1 isotype.

Anti-CD40 mAb therapy alters the antigen specific T cell cytokine profile.

The increase of anti-CII IgG1/IgG2a ratio, observed after anti-CD40 mAbs treatment, suggests that anti-CD40 mAb therapy may have altered the T lymphocyte Th1/Th2 balance, therefore we studied the expression of Th1 (IFN- γ) and Th2 (IL-4 and IL-5) cytokines in the different groups of mice. CII re-stimulation of splenocyte cultures (SPC) obtained from mice treated *in vivo* with 3\23 showed a significant inhibition of IFN- γ (Figure 6a), whereas the levels of IL-5 production were increased relative to the pattern observed in the isotype control treated mice (Figure 6c).

In SPC from FGK45 treated mice a tendency to inhibit IFN- γ was observed, but no IL-5 production was detected (Figure 6a, c). Under these experimental conditions IL-4 was not detected in any of the samples studied (Figure 6b).

We also tested whether *in vitro* manipulation with the anti-CD40 mAb could influence the Th1/Th2 balance. In these experimental conditions *in vitro* re-stimulation of SPC from AFRC-MAC-1 treated mice (controls) with CII and 3\23, led to a significant reduction of IFN- γ production (Figure 6d) and to an increase of IL-5 and for the first time detection of IL-4 (Figure 6e and f). Of interest was the finding that the *in vitro* addition of 3\23 to SPC from 3\23 treated mice, further reduced the IFN- γ production (Figure 6d), paralleled by an increase in the IL-5 and more remarkably IL-4 levels (Figure 6e, f), compared to the value obtained from *in vivo* AFRC-MAC-1 treated SPC. *In vitro* challenge of SPC from AFRC-MAC-1 treated mice with FGK45 reduced the IFN- γ production (6d) and up-regulated IL-5 production (6f). These results indicate that agonistic anti-CD40 mAb therapy can effectively alter a pathogenic antigen- specific T cell phenotype and redirect, particularly in the case of 3\23, the auto- aggressive pro-inflammatory Th1 response into an anti-inflammatory Th2 response.

Agonistic anti-CD40 administration before immunisation does not prevent CCIA.

It has recently been established that stimulation with agonistic anti-CD40 mAb can enhance T cell-independent¹³ and T cell dependent immune responses¹⁸. Since our data were in apparent disagreement with several well-established studies, we speculated that it was the timing of anti-CD40 mAb administration, which could be the crucial factor for the kind of immune response observed. Indeed the studies in which CD40 stimulation proved to amplify the immune response, the agonistic anti-CD mAb was introduced prior to¹⁸ or at¹³ the time of immunisation. To establish whether administration of anti-CD40 mAb before CII immunisation influenced the outcome of the disease we treated mice with 5 daily injections (300 μ g/mouse) of FGK45, 3\23, AFRC-MAC-1 or PBS (300 μ l/mouse) prior to CII in CFA immunisation. Thus we implemented an experimental protocol even more stringent to the one used by others prior immunisation^{13, 18}. In these experimental

conditions both FGK45 and 3\23 did not prevent the induction of CCIA (Figure 7). Thus indicating, in our experimental model, that the timing of the administration of anti-CD40 mAbs appears to be crucial for the outcome of its effect.

Figure 8 shows that anti-CD40 antibodies reduce Ig G2a (TH1 pathogenic) levels in favour of Ig G1 (TH2) in splenocyte. Cells were treated with AFRC mac1 isotype monoclonal antibody or anti-CD40 monoclonal antibodies. The cells were then placed in SCID mice for one month before assaying for Ig G2a or Ig G1.

Figure 9 shows the effect of injecting anti-CD40 antibodies into mice on IL-12 productions in pathogenic/arthritisogenic splenocytes.

DISCUSSION

Disruption of the CD40-CD154 axis has recently gained central stage as one of the ideal approaches to control unwanted immune responses⁶, more interestingly even other therapeutic strategies, such as the use of non depleting anti-CD4 mAb, seem to be based on down-modulation of CD154 expression (unpublished results). Clearly the engagement of CD40 by agonistic mAbs provides the opposite effect of boosting the immune response, a property widely used to enhance the immune system when required^{13-16, 18}. However, in the course of earlier studies with non-depleting anti-CD4 mAb, the inventors paradoxically observed that agonistic anti-CD40 mAb could successfully prevent CCIA (unpublished results).

The results described here further support and expand this surprising finding establishing the therapeutic effect of agonistic anti-CD40 mAb in an autoimmune inflammatory animal model of RA. It is most remarkable that the agonistic anti-CD40 mAb therapy seems to be extremely effective in controlling the progression of ongoing CCIA. In our experimental model we could detect that a predominant CII specific Th2 response was induced in the anti-CD40 mAb treated mice suggesting that one of the mechanisms instigated by agonistic anti-CD40 mAb involves the induction of an autoantigen specific Th2 response.

One possible explanation for this distinct change in the profile of the T cell response, from a Th1 to a Th2, is based on the potential shift of the cell type which acts as the dominant antigen presenting cell (APC) driving the expansion and activation of CII specific T cells. It is well established that presentation of antigen to T cells by DC tend to lead, in particular in the presence of 'danger'²⁴, to a Th1 type of response²⁵. On the contrary, if non-resting B cells act as APC they induce the activation of T cells expressing IL-4 with a Th2 compatible cytokine profile²⁶⁻²⁸. The induction of these Th2 responses have been well established to antagonise cell mediated immune responses²⁹⁻³¹. Moreover, administration of Myelin Basic protein (MBP) peptide covalently linked to anti-IgD, to force presentation of the immunogenetic peptide by B cells, prevented the induction of a cell-mediated

autoimmune disease in rats³². This prevention was subsequently shown to be due to a protective Th2 response and an alteration in the migratory properties of autoantigen specific T lymphocytes³³. In this context it is essential to note that agonistic anti-CD40 mAb treated mice show a dramatic expansion of B cells and that a large proportion of these B cells are likely to be CII specific as large amounts of CII specific antibodies are present in these mice. Therefore the overwhelming APC population in the anti-CD40 mAb treated mice are likely to be the non-resting CII specific B cells. In addition it has to be reminded that antigen specific B cells are probably the most efficient APC, in terms of capturing ability³⁴, thus two factors, large expansion of non-resting B cells, and their antigen specificity would synergise and completely alter the balance of CII presenting APCs. It is also important to note in this respect that CD40 engagement stimulates B cell antigen processing and hence enhance their APC function³⁵. Further supporting the hypothesis that agonistic anti-CD40 mAb manipulation, during an ongoing autoimmune chronic inflammatory process, can favourably redirect the type of immunological response to the driving autoantigen.

It is, however, apparent from our study that a simple switch from a Th1 to a Th2 type of response could not account for the entire therapeutic action of anti-CD40 mAb. Firstly, the rapid effect after injection of agonistic anti-CD40 mAb seems to be unlikely only related to a switch of the dominant APC population. Secondly, the subtle but persistent differences between the effects of FGK45 and 3\23 indicate that alternative as yet undefined therapeutic mechanisms act in our model. In addition, the apparently least powerful Th2 inducer, FGK45, displays a potent therapeutic effect, possibly more so than that of 3\23. These discrepancies between the two antibodies, which were shown to recognise different epitopes on CD40 (data not shown), indicate that fine differences between these reagents create a distinct functional activity.

However, the paradox of the dual ability of agonistic anti-CD40 mAb to enhance immune responses, which proved to enforce a protective immune response to cancer¹⁴⁻¹⁶ and infectious agents¹³, as well as control ongoing inflammatory autoimmune processes, as

reported here, remains to be reconciled. We suggest that the timing of administration might be the key to explain such a difference, a view supported by others¹⁸. The inventors have shown that the administration of anti-CD40 mAb before immunisation, compared to after antigenic challenge, does not produce the same functional outcome. This differential action of anti-CD40 mAb might be a good presage for the application of anti-CD40 mAbs to fight infections or cancer and alternatively, to treat chronic autoimmune inflammatory processes. Indeed in the first case the treatment will be properly timed prior to or at the time of antigenic challenge aiming to support the initiating response whilst in the second case we would interfere with an ongoing process aiming to deviate the established antigenic perception.

In conclusion, the inventors have described the totally unexpected finding that agonistic anti-CD40 mAbs can successfully control chronic autoimmune inflammatory processes opening their potential use for patients suffering from autoimmune disorders for whom to date no satisfactory therapy is available.

The results demonstrate that agonistic anti-CD40 antibodies are effective in controlling immune responses when there is already an immune response in place. For example, in immune-inflammatory responses in rheumatoid arthritis and other chronic autoimmune diseases.

REFERENCES

1. Bluestone, J.A., New perspectives of CD28-B7-mediated T cell costimulation. *Immunity* **2**, 555-9 (1995).
2. Lenschow, D.J. *et al.* Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. *Science* **257**, 789-92 (1992).
3. Lin, H. *et al.* Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4Ig plus donor-specific transfusion. *J. Exp. Med.* **178**, 1801-6 (1993).
4. Maiuri, L. *et al.* Blockage of T-cell costimulation inhibits T-cell action in celiac disease. *Gastroenterology* **115**, 564-72 (1998).
5. Guinan, E.C. *et al.* Transplantation of anergic histoincompatible bone marrow allografts. *N. Engl. J. Med.* **340**, 1704-14 (1999).
6. Grewal, I.S. & Flavell, R.A. CD40 and CD154 in cell-mediated immunity. *Annu. Rev. Immunol* **16**, 111-35 (1998).
7. Durie, F.H. *et al.* Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40. *Science* **261**, 1328-30 (1993).
8. Mohan, C., Shi, Y., Laman, J.D. & Datta, S.K. Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. *J. Immunol* **154**, 1470-80 (1995).
9. Griggs, N.D. *et al.* The relative contribution of the CD28 and gp39 costimulatory pathways in the clonal expansion and pathogenic acquisition of self-reactive T cells. *J. Exp. Med.* **183**, 801-10 (1996).

10. Balasa, B. *et al.* CD40 ligand-CD40 interactions are necessary for the initiation of insulinitis and diabetes in nonobese diabetic mice. *J. Immunol* **159**, 4620-7 (1997).
11. Howard, L.M. *et al.* Mechanisms of immunotherapeutic intervention by anti- CD40L (CD154) antibody in an animal model of multiple sclerosis. *J. Clin. Invest* **103**, 281-90 (1999).
12. Kirk, A.D. *et al.* Treatment with humanised monoclonal antibody against CD154 prevents acute renal allograft rejection in non-human primates. *Nat. Med.* **5**, 686-93 (1999).
13. Dullforce, P., Sutton, D.C. & Heath, A.W. Enhancement of T cell-independent immune responses *in vivo* by CD40 antibodies. *Nat. Med.* **4**, 88-91 (1998).
14. French, R.R., Chan, H.T., Tutt, A.L. & Glennie, M.J. CD40 antibody evokes a cytotoxic T-cell response that eradicates lymphoma and bypasses T-cell help. *Nat. Med.* **5**, 548-53 (1999).
15. Diehl, L. *et al.* CD40 activation *in vivo* overcomes peptide-induced peripheral cytotoxic T-lymphocyte tolerance and augments anti-tumor vaccine efficacy. *Nat. Med.* **5**, 774-9 (1999).
16. Sotomayor, E.M. *et al.* Conversion of tumor-specific CD4+ T-cell tolerance to T-cell priming through *in vivo* ligation of CD40. *Nat. Med.* **5**, 780-7 (1999).
17. Mauri, C., Chu, C.Q., Woodrow, D., Mori, L. & Londei, M. Treatment of a newly established transgenic model of chronic arthritis with nondepleting anti-CD4 monoclonal antibody. *J. Immunol* **159**, 5032-41 (1997).
18. Maxwell, J.R., Campbell, J.D., Kim, C.H. & Vella, A.T. CD40 activation boosts T cell immunity *in vivo* by enhancing T cell clonal expansion and delaying peripheral T cell deletion. *J. Immunol* **162**, 2024-34 (1999).

19. Taylor, P.C., Plater-Zyberk, C. & Maini, R.N. The role of the B cells in the adoptive transfer of collagen-induced arthritis from DBA/1 (H-2q) to SCID (H-2d) mice. *Eur. J. Immunol* **25**, 763-9 (1995).
20. Liblau, R.S., Singer, S.M. & McDevitt, H.O. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* **16**, 34-8 (1995).
21. Abbas, A.K. Murphy, K.M. & Sher, A. Functional diversity of helper T lymphocytes. *Nature* **383**, 787-93 (1996).
22. Chu, C.Q. & Londei, M. Induction of Th2 cytokines and control of collagen- induced arthritis by non-depleting anti-CD4 Abs. *J. Immunol* **157**, 2685-9 (1996).
23. Rabinovich, G.A. *et al.* Recombinant galectin-1 and its genetic delivery suppress collagen-induced arthritis via T cell apoptosis. *J. Exp. Med.* **190**, 385-98 (1999).
24. Matzinger, P. Tolerance, danger, and the extended family. *Annu. Rev. Immunol* **12**, 991-1045 (1994).
25. Cella, M., Sallusto, F. & Lanzavecchia, A. Origin, maturation and antigen presenting function of dendritic cells. *Curr Opin Immunol.* **9**, 10-6 (1997).
26. Finkelman, F.D. *et al.* Production of BSF-1 during an *in vivo*, T-dependent immune response. *J. Immunol* **137**, 2878-85 (1986).
27. Finkelman, F.D., Snapper, C.M., Mountz, J.D. & Katona, I.M. Polyclonal activation of the murine immune system by a goat antibody to mouse IgD. IX. Induction of a polyclonal IgE response. *J. Immunol* **138**, 2826-30 (1987).
28. Gajewski, T.F., Pinnas, M., Wong, T. & Fitch, F.W. Murine Th1 and Th2 clones proliferate optimally in response to distinct antigen-presenting cell populations. *J. Immunol* **146**, 1750-8 (1991).

29. Powrie, F., Menon, S. & Coffman, R.L. Interleukin-4 and interleukin-10 synergize to inhibit cell-mediated immunity *in vivo*. *Eur. J. Immunol* **23**, 3043-9 (1993).
30. Sadick, M.D. *et al.* Cure of murine leishmaniasis with anti-interleukin 4 monoclonal antibody. Evidence for a T cell-dependent, interferon gamma-independent mechanism. *J. Exp. Med.* **171**, 115-27 (1990).
31. Fiorentino, D.F., Bond, M.W. & Mosmann, T.R. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* **170**, 2081-95 (1989).
32. Day, M.J., Tse, A.G., Puklavec, M., Simmonds, S.J. & Mason, D.W. Targeting autoantigen to B cells prevents the induction of a cell-mediated autoimmune disease in rats. *J. Exp. Med.* **175**, 655-9 (1992).
33. Saoudi, A., Simmonds, S., Huitinga, I. & Mason, D. Prevention of experimental allergic encephalomyelitis in rats by targeting autoantigen to B cells: evidence that the protective mechanism depends on changes in the cytokine response and migratory properties of the autoantigen-specific T cells. *J. Exp. Med.* **182**, 335-44 (1995).
34. Lanzavecchia, A. Antigen-specific interaction between T and B cells. *Nature* **314**, 537-9 (1985).
35. Faassen, A.E., Dalke, D.P., Berton, M.T., Warren, W.D. & Pierce, S.K. CD40-CD40 ligand interactions stimulate B cell antigen processing. *Eur. J. Immunol* **25**, 3249-55 (1995).
36. Mori, L., Loetscher, H., Kakimoto, K., Bluethmann, H. & Steinmetz, M. Expression of a transgenic T cell receptor beta chain enhances collagen-induced arthritis. *J. Exp. Med.* **176**, 381-8 (1992).

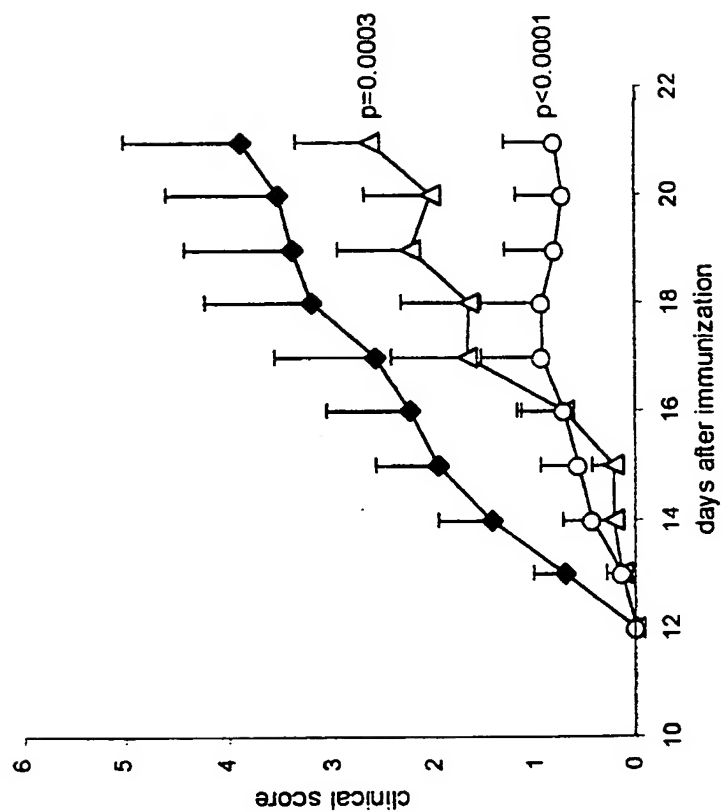
37. Hasbold, J., Johnson-Leger, C., Atkins, C.J., Clark, E.A. & Klaus, G.G. Properties of mouse CD40: cellular distribution of CD40 and B cell activation by monoclonal anti-mouse CD40 antibodies. *Eur. J. Immunol* **24**, 1835-42 (1994).
38. Rolink, A., Melchers, F. & Andersson, J. The SCID but not the RAG-2 gene product is required for S mu-S epsilon heavy chain class switching. *Immunity* **5**, 319-30 (1996).
39. Miller, E.J. Structural studies on cartilage collagen employing limited cleavage and solubilization with pepsin. *Biochemistry* **11**, 4903-9 (1972).
40. Mauri, C., Williams, R.O. Walmsley, M. & Feldmann, M. Relationship between Th1/Th2 cytokine patterns and the arthritogenic response in collagen-induced arthritis. *Eur. J. Immunol* **26**, 1511-8 (1996).
41. Williams, R.O. Williams, D.G. & Maini, R.N. Anti-type II collagen ELISA. Increased disease specificity following removal of anionic contaminants from salt- fractionated type II collagen. *J. Immunol Methods* **147**, 93-100 (1992).

Claims

1. Use of an agonistic CD40 binding protein in the manufacture of a medicament to treat an autoimmune disease.
2. A method of treating an autoimmune disease comprising administering to an animal or patient a pharmaceutically effective dose of an agonistic CD40 binding protein.
3. A use or method according to claim 1 or 2, wherein the autoimmune disease is selected from rheumatoid arthritis, systemic lupus erythematosus, diabetes myellitus and multiple sclerosis.
4. A method or use, according to any preceding claim, wherein the agonistic CD40 binding protein is an anti-CD-40 antibody.
5. A method or use according to claim 4, wherein the anti-CD40 antibody is a polyclonal antibody or a monoclonal antibody.
6. A method or use according to claim 5, wherein the antibody is a monoclonal antibody with the functional characteristics of FGK45 or 3\23, when implemented in man, or CD154.
7. A method of studying an autoimmune disease comprising administering a pharmaceutically effective dose of an agonistic CD40 binding protein to an animal, sample of tissue, or cell and observing a physiological change to the animal, sample of tissue or cell.
8. A method according to claim 7, wherein the CD40 binding protein is an anti-CD40 antibody.
9. A method according to claims 7 or 8, wherein the method is carried out *in vitro* on an isolated cell, cultured cell, or an isolated sample of tissue.

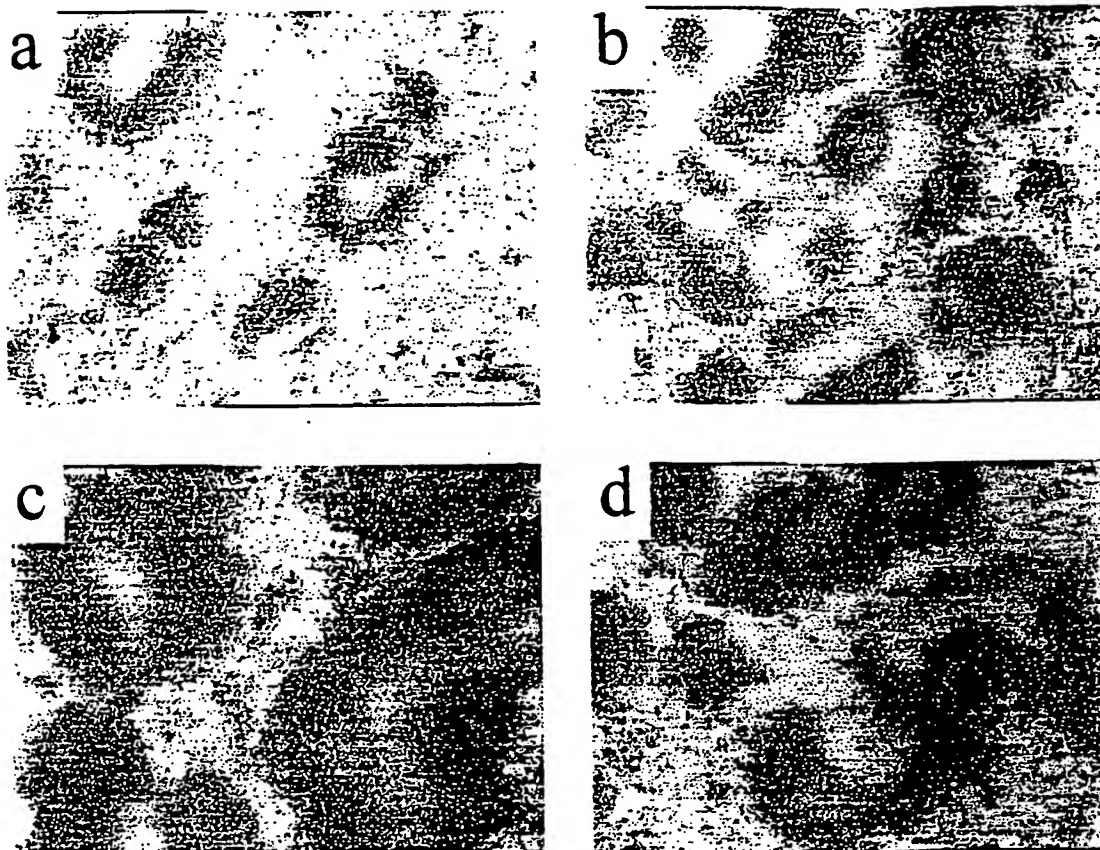
1/9

Figure 1



2/9

Figure 2



3/9

Figure 3

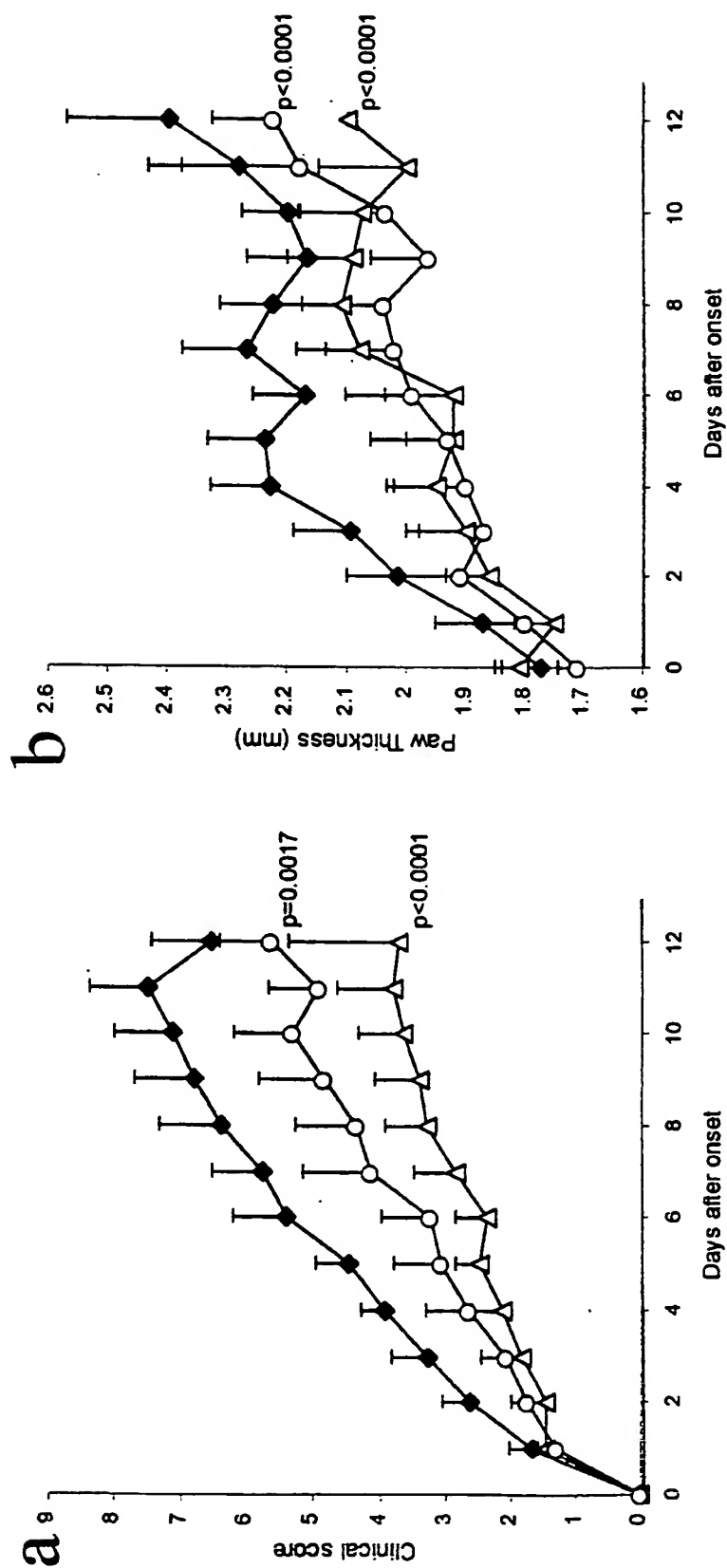
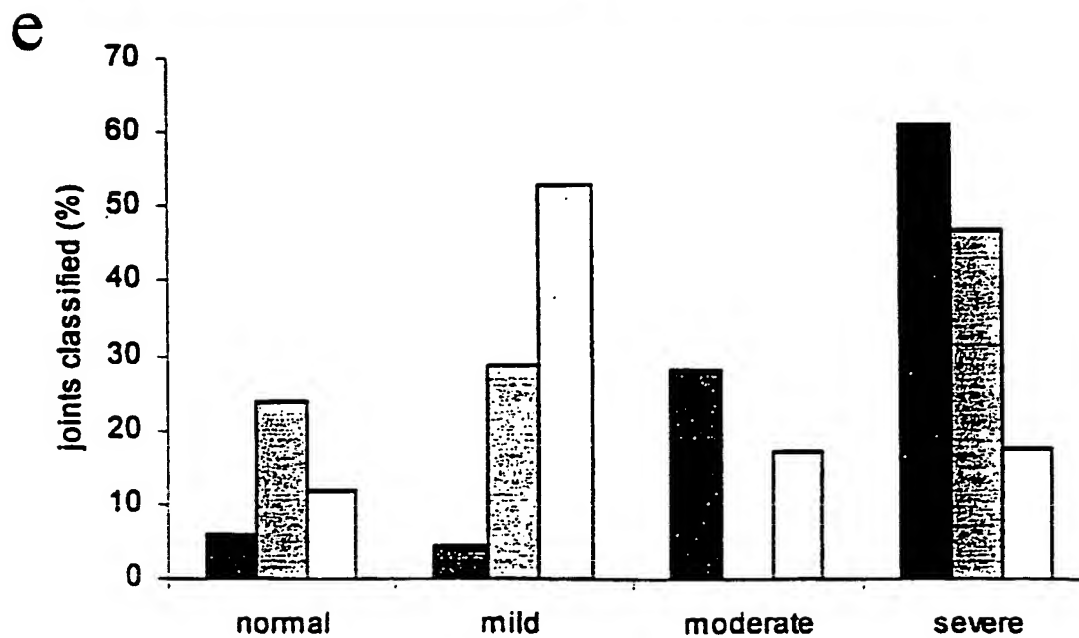
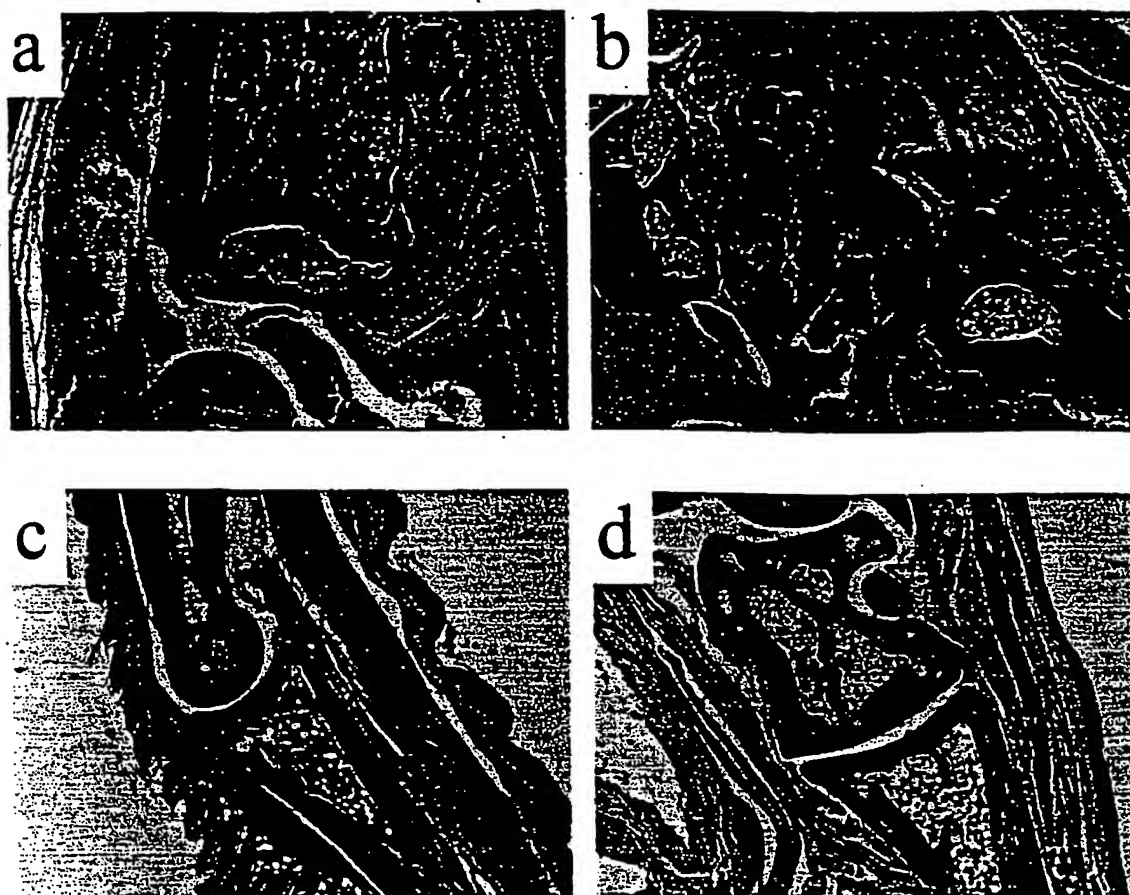
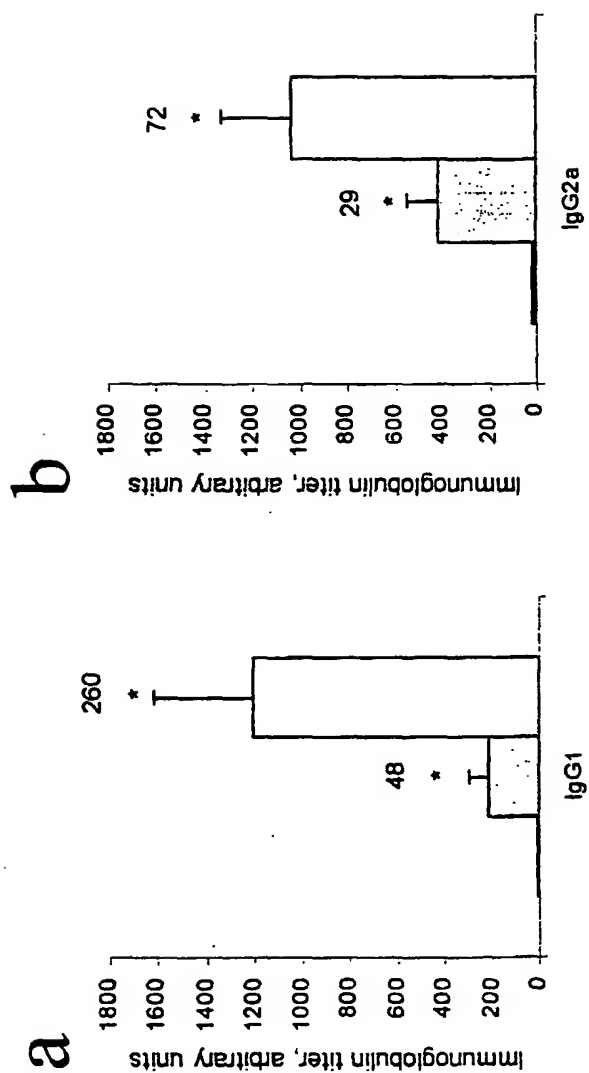


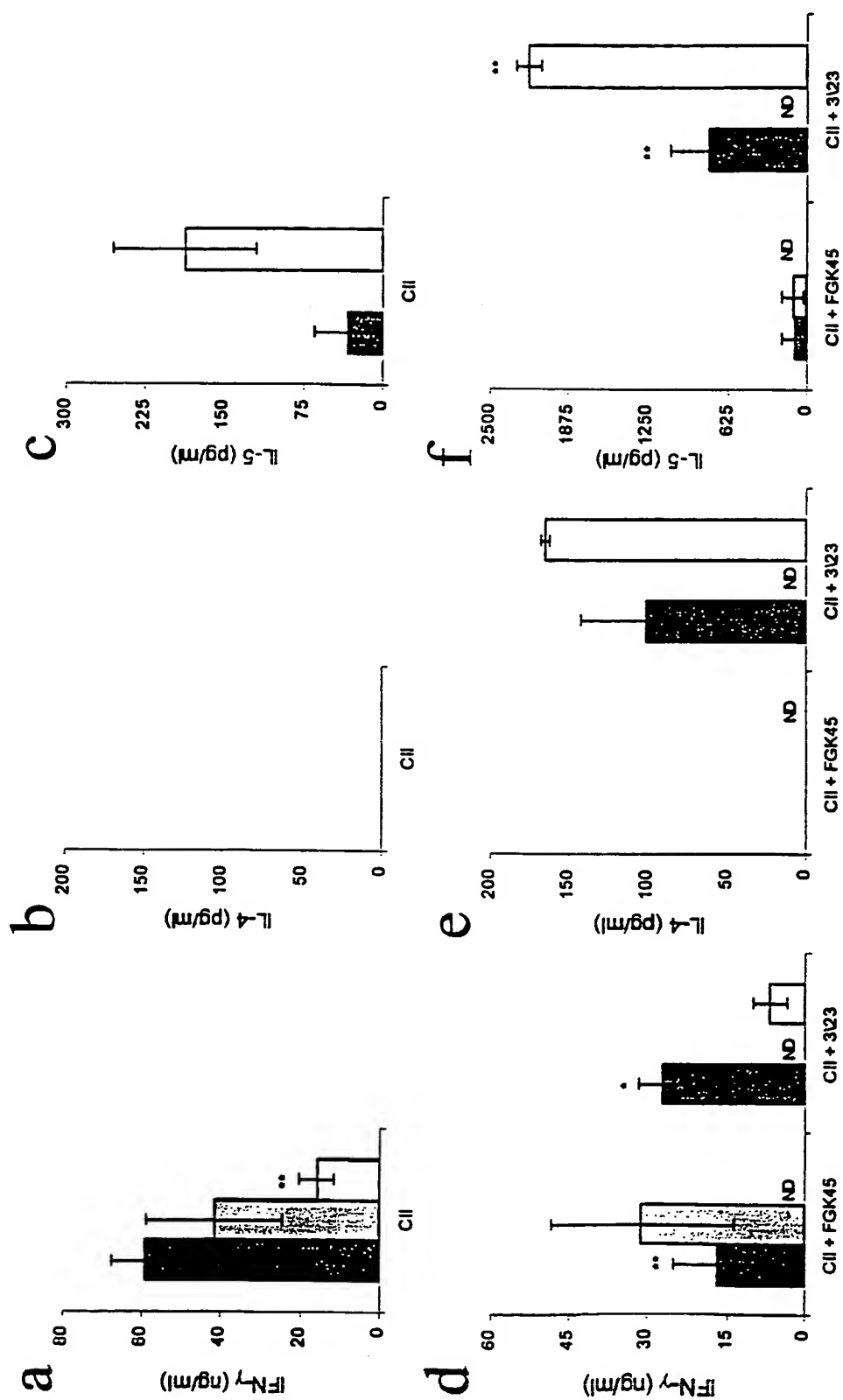
Figure 4

5/9

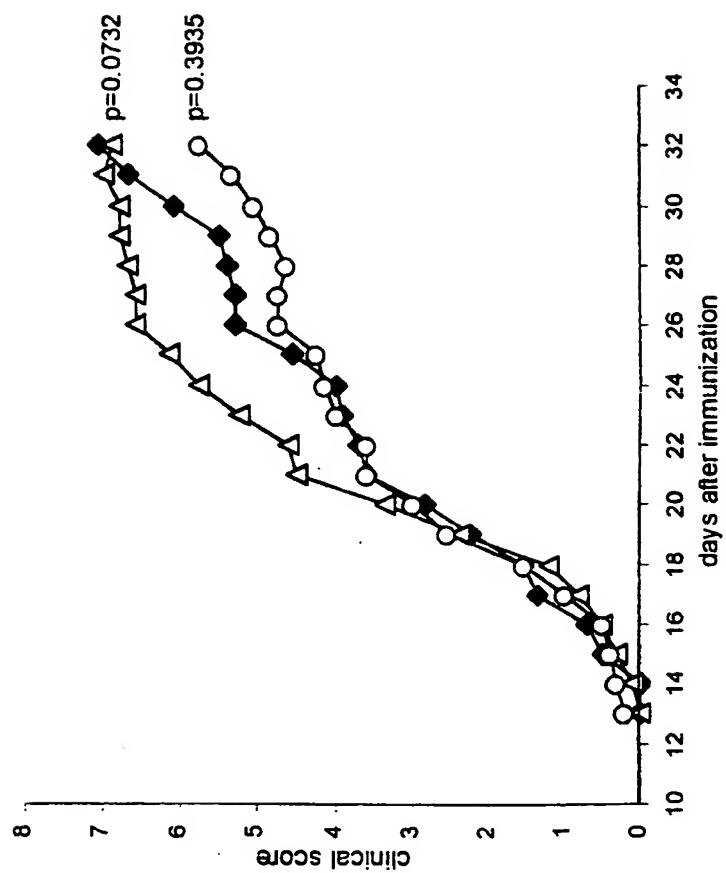
Figure 5

6/9

Figure 6

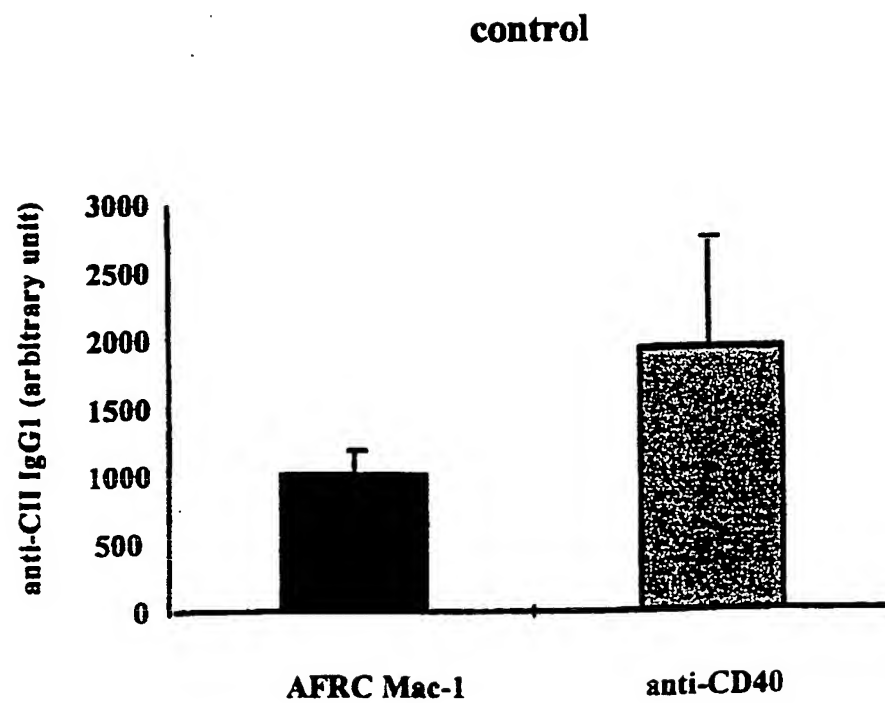
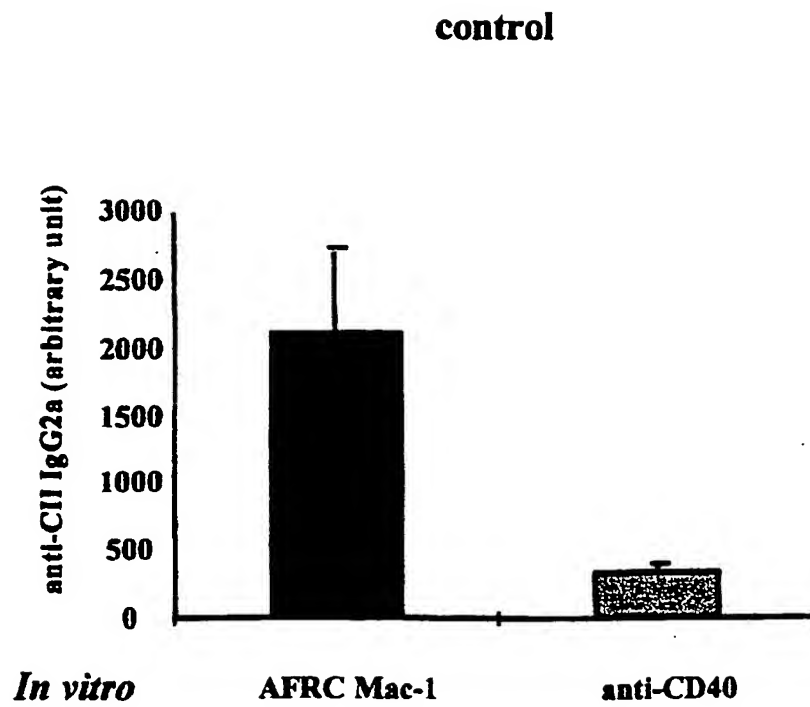


7/9

Figure 7

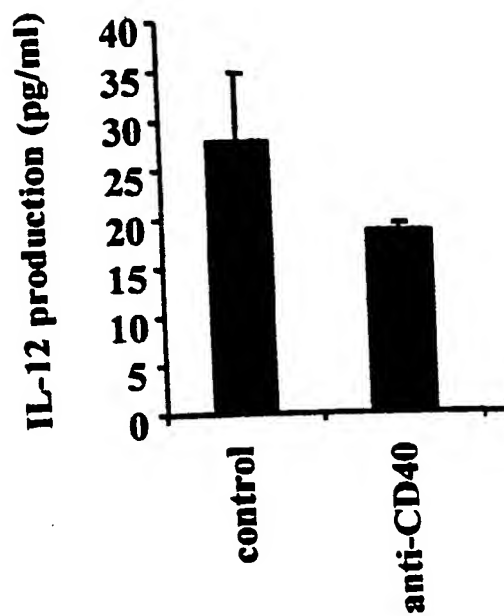
8/9

Figure 8



9/9

Figure 9



INTERNATIONAL SEARCH REPORT

Int. l. Application No

PCT/GB 00/04511

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K39/395 G01N33/53 G01N33/577 A61P37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EMBASE, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M. DEN HARTOG ET AL.: "Generation of a humanized anti-CD40 mab for treatment of autoimmune diseases." IMMUNOTECHNOLOGY, vol. 2, no. 4, 1 November 1996 (1996-11-01), page 299 XP002032468 Amsterdam, The Netherlands page 299, left-hand column ---	1-9
A	M. ROSKROW ET AL.: "Autoimmune disease induced by dendritic cell immunization against leukemia." LEUKEMIA RESEARCH, vol. 23, 1999, pages 549-557, XP000943365 Oxford, GB abstract discussion --- -/--	1-9

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

S document member of the same patent family

Date of the actual completion of the international search

5 February 2001

Date of mailing of the international search report

21/02/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Nooij, F

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/04511

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	R. FRENCH ET AL.: "CD40 antibody evokes a cytotoxic T-cell response that eradicates lymphoma and bypasses T-cell help." NATURE MEDICINE, vol. 5, no. 5, May 1999 (1999-05), pages 548-553, XP002159390 New York, NY, USA abstract page 552, right-hand column, line 11 - line 17 ---	1-9
A	WO 93 08207 A (IMMUNEX CORP.) 29 April 1993 (1993-04-29) example 12 claim 15 ---	1-9
A	K. KATO ET AL.: "The soluble CD40 ligand sCD154 in systemic lupus erythematosus." THE JOURNAL OF CLINICAL INVESTIGATION, vol. 104, no. 7, October 1999 (1999-10), pages 947-955, XP002159391 New York, NY, USA abstract ---	1-9
A	WO 98 30241 A (BIOGEN INC.) 16 July 1998 (1998-07-16) examples claims ---	1-9
P,X	C. MAURI ET AL.: "Therapeutic activity of agonistic monoclonal antibodies against CD40 in a chronic autoimmune inflammatory process." NATURE MEDICINE, vol. 6, no. 6, June 2000 (2000-06), pages 673-679, XP002157458 New York, NY, USA the whole document ---	1-9
P,X	E. ZANELLI ET AL.: "A dual function for CD40 agonists." NATURE MEDICINE, vol. 6, no. 6, June 2000 (2000-06), pages 629-630, XP002159392 New York, NY, USA the whole document ---	1-9

-/-

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 00 04511

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.1

Although claims 2 (completely) and 3-6 (all partially, as far as an in vivo method is concerned) are directed to a method of treatment of the human/animal body, and although claims 7 and 8 (both partially, as far as an in vivo method is concerned) are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Int. Patent Application No.

PCT/GB 00/04511

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>C. MAURI ET AL.: "Therapeutic activity of agonistic anti-CD40 mAb in a chronic autoimmune inflammatory process." THE FASEB JOURNAL, vol. 14, no. 6, 20 April 2000 (2000-04-20), page A1101 XP002159393 Bethesda, MD, USA abstract 110.2</p> <p style="text-align: center;">-----</p>	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. l. Application No

PCT/GB 00/04511

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9308207 A	29-04-1993	AU 661360 B	20-07-1995
		AU 3122693 A	21-05-1993
		CA 2121798 A	29-04-1993
		EP 0667901 A	23-08-1995
		EP 0897983 A	24-02-1999
		FI 941837 A	30-05-1994
		FI 981765 A	17-08-1998
		JP 2877788 B	31-03-1999
		JP 10150994 A	09-06-1998
		JP 7504083 T	11-05-1995
		NO 941422 A	27-06-1994
		NO 980030 A	05-01-1998
		US 6087329 A	11-07-2000
		US 5961974 A	05-10-1999
		US 5716805 A	10-02-1998
		US 5981724 A	09-11-1999
		US 5962406 A	05-10-1999
WO 9830241 A	16-07-1998	AU 721697 B	13-07-2000
		AU 5735398 A	03-08-1998
		BR 9807471 A	21-03-2000
		CN 1248921 T	29-03-2000
		CZ 9902443 A	13-10-1999
		EP 0966302 A	29-12-1999
		NO 993275 A	09-09-1999
		PL 334500 A	28-02-2000
		AU 5623398 A	22-09-1998
		HU 0001263 A	28-08-2000
		WO 9839026 A	11-09-1998